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UPDATED MODIFICATIONS ABOUT SPIN∞ BIOREACTOR

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***Abstract*— This paper carries out the implementation of a Raspberry-controlled bioreactor. The previously proposed model was analyzed and assembled to determine its effectiveness and solutions to the problems that emerged during construction were highlighted. The realization of the project is possible, as now some elements can be replaced. Overall, these modifications on this miniature rotary bioreactor make the apparatus exceptionally easy to build following designated guidelines. This updated device is susceptible to various experiments of varying lengths without having to worry about possible malfunctions such as unexpected events or hardware and software problems.**

Keywords— **Bioreactors, 3D Model, Organoid, Tissue Engineering, 3D printing.**

I. INTRODUCTION

The term Bioreactor[1] in recent years is becoming more and more varied, representing heterogeneous devices, but united and similar to each other thanks to the ability to conduct biochemical and biological processes in environmental and operational conditions meticulously monitored and controlled by software. Bioreactors are all those devices designed and built in research laboratories in many parts of the world to conduct dynamic cell cultures, thus stimulating the cultured cells by applying controlled and reproducible biophysical stimuli[2]. In terms of EU regulation, bioreactors are defined as numerous devices developed at the request of the clinical world for the implementation of bioprocesses in the field of advanced therapies such as: Cell Therapy, Gene Therapy and Tissue Engineering[3]. The bioreactors, often conceived and developed by the researchers themselves, have unique characteristics that, starting from the available laboratory equipment, continue to play a key role in the engineering processes of biological tissues and in the implementation of 3D cell cultures, increasing their use by NAM's[4]. Many advances related to the use of bioreactors for the generation of biological constructs in vitro, would not have been possible without the advances made in the field of biomaterials, where technologies such as 3D printing in the context of rapid prototyping have allowed the development of innovative devices in able to respond to the multiple needs of individual researchers and departments. Despite various successes in the engineering of biological tissues[5] numerous obstacles remain to be overcome for the reproduction of constructs in which three-dimensionality and mechanics play relevant roles. One of these causes is to be found in the difficulties of generating and maintaining cell cultures in three dimensions in vitro, determining a correct structural organization of the development target. Functional tissue engineering proposes to reproduce in vitro the complex of stresses to which tissues are subjected during in vivo development, in order to determine a functional structure similar to the native one in the engineered tissues[5][6]. The application of physical stimuli, by altering the distribution and organization of structural elements inside and outside the cells, determines cellular physiology[8]. Therefore, bioreactors intended as systems for the application of controlled and reproducible biophysical stimuli represent an essential tool for the creation of functional districts, helping to overcome the limits imposed by static 2D monolayer cultures [9].

Recently, several Bioreactors have been released but in particular a device called Spinfinity (Spin∞). This bioreactor is customized for organoid culture via miniaturization and increased productivity. The device has a small footprint and an integrated rotation mechanism that does not require a large amount of space in the dedicated incubator[10].

II. PROPOSED MODIFICATION

A. Analysis of the original measurements of the prototype

To improve this device, first the analysis of the device was carried out and then the purchase of some components indicated with the 3D printing of the parts. During the assembly of the physical components and the subsequent use of the software, we realized some weaknesses and some flaws that were not described in the construction protocol Fig. 1.

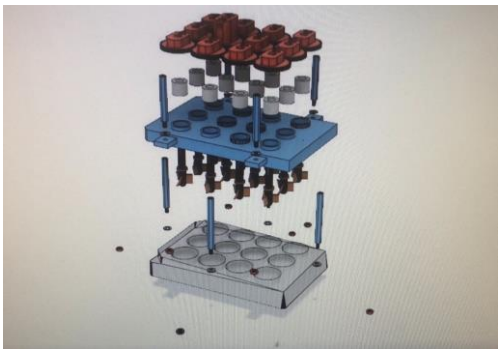

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Fig. 1. Decomposition of the Spin∞ model: through the original video you can see some passages

First of all, some sizes for screws and bolts were too small to be able to find them and in order not to spend too much on the shipment of materials that can be found elsewhere, a larger size was opted for. This step was not only fundamental for the success of the protocol, but it gave greater stability to the device giving a better rotational function of the assembled gears Fig. 2.

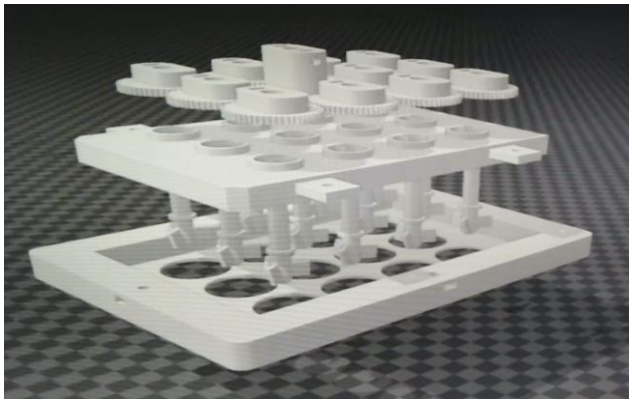

Fig. 2. Upload of the prototype to the 3D viewer with structural measurements and changes made to improve the use of the device

Next, we considered the basic bioreactor design. Due to the original Base and 12-Well Plate Lid file shape problem, the normally used culture plate could not be inserted as it did not match. These parts also have the benefit of being 3D printed as separate components, and the updated design now allows for better handling and ease of use Fig. 3.

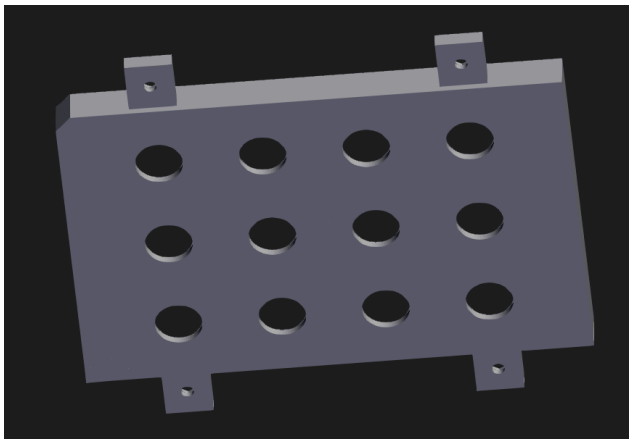

Fig. 3. Creation of 12 Well Plate Lid with compatible angle to the 12 for Well Plate available in the laboratory

B. ABS alternative 3D printing filament

Regarding the printing material, originally the resin was used as proposed but it was seen that filaments in acrylonitrile-butadiene-styrene (ABS) copolymers could be used. The ABS show excellent toughness and good dimensional stability and high impact resistance[11]. This widespread filament requires a plate heated to 90 ° -115 ° to adhere and another setting parameter for the printer such as the nozzle temperature equal to 215 ° -250 °[12]. For culturing, sequential washing with 10% bleach, 70% ethanol, rinsing with distilled water and UV irradiation under a fume hood are required to make the product sterile as reported in the original protocol. Some precautions, as already clarified above, are essential to obtain the perfect first layer as it is the most important part of the print Fig. 4. The additional rules for success are the print bed must be leveled, extrude the first layer at the correct height of the bed, use the RAFT function in the slicing program. The function allows you to create a base, as high as we want, so that the bottom face of the object is not attached to the print surface. Adhesion to the print bed requires a high surface temperature but the high temperature has undesirable effects creating a warped pattern. Thanks to RAFT it is therefore possible to move away from the hot printing plate ensuring a greater adhesion surface Fig. 5. Depending on the printer, the correct temperature must be set. If the temperature is too low, the extruded filament will not stick in a homogeneous way, resulting in a fragile and delaminated piece. On the contrary, if the temperature is too high, the filament will tend to run between one wall and the other, leaving threads and the layers tend to be crushed.

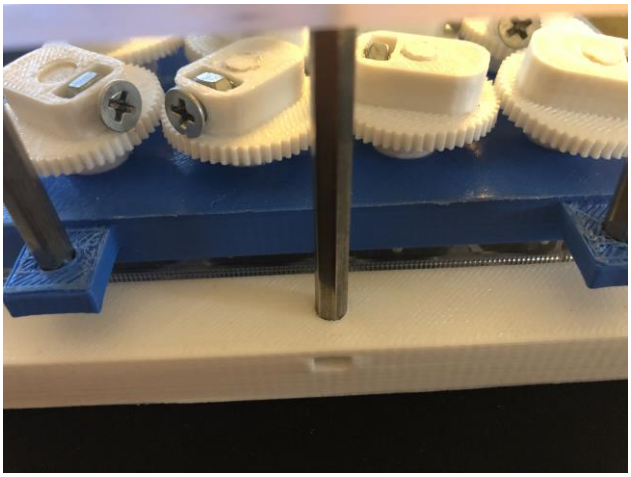

Fig. 4. ABS as an alternative 3D printing filaments: the lower white object is the Base 4 mm V.2.stl model; while in the center there is a blue plate model 12 wells 4mm V.2.stl; finally above there are the Motor Gear 4mm X 11 V.2.stl

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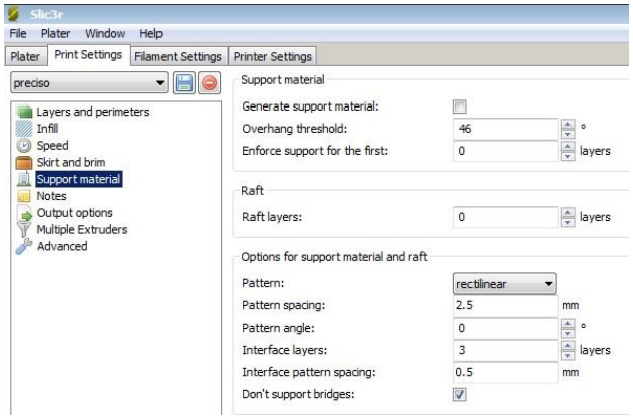
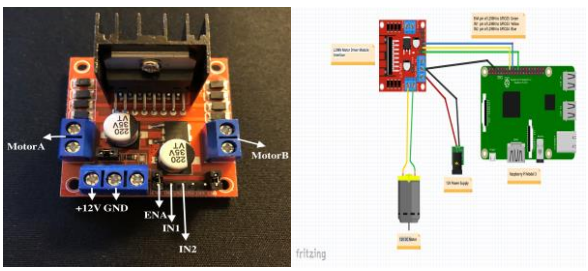


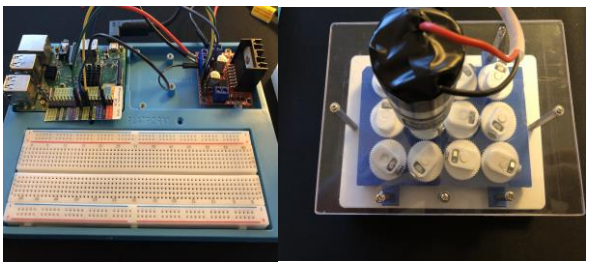
Fig. 5. RAFT function on Slic3r and some parameters such as pattern: allows you to choose the texture of the raft; pattern spacing: change the density of the texture; pattern angle: allows you to change the direction of the raft lines; interface layers: this value allows you to make solid layers before the base of our object.

III. EXPERIMENT AND RESULT

The procedure for the assembly and operation of the Spin∞ bioreactor is described in its original protocol [10], but some figures have been reported here to show some steps that were not considered during the assembly. Fig. 6 shows all the components. Further details on bioreactor hardware and all components are provided in the Table 1. The proposed script was tested using the DC Motor normally. Following the online script guidelines, it was decided to use and modify a script, adapting it to the needs of the bioreactor. From the simulation of the results of the experiment, we can draw the conclusion that this method is robust and is able to perform its task.



(a) (b)



(c) (d)

Fig. 6. (a) L298N Motor Driver Module with names of all the components on it. (b) Shows the circuit diagram of the Raspberry Pi L298N Motor Driver Module Interface. The circuit diagram is made with the help of Fritzing App. (C) The design of the Raspberry Pi L298N Motor Driver Interface Circuit (d) Fully assembled bioreactor.

Table -1 Design files

	File type	Location of the file
Spinning Alpha Bioreactor V.2.stl	**STL**	**https://grabcad.com/library/bioreactor-8/details?folder_id=7923624**
12-Well Plate 4mm V.2.stl	**STL**	**https://grabcad.com/library/bioreactor-8/details?folder_id=7923624**
CCW Paddle Full X6 V.2.stl	**STL**	**https://grabcad.com/library/bioreactor-8/details?folder_id=7923624**
Motor Gear 4mm X11 V.2.stl	**STL**	**https://grabcad.com/library/bioreactor-8/details?folder_id=7923624**
Base 4mm V.2.stl	**STL**	**https://grabcad.com/library/bioreactor-8/details?folder_id=7923624**
CW Paddle Full X6 V.2.stl	**STL**	**https://grabcad.com/library/bioreactor-8/details?folder_id=7923624**
Motor Shaft Gear V.2.stl	**STL**	**https://grabcad.com/library/bioreactor-8/details?folder_id=7923624**
Spinning Bioreactor Alpha .py	**PY**	**https://github.com/ArnaudCapuzzo/Spinning-Bioreactor-Alpha-.git**

Table 1 contains the STL design files for the 3D printed parts with the attached site, plus a new Python script for running the bioreactor and a video demonstration of the step-by-step assembly of the device has been included.

IV. CONCLUSION

Bioreactors substantially used for research purposes prove to be indispensable tools for the generation, maintenance and study of 3D cellular constructs; but have characteristics that are difficult to translate to industrial use such as for the production of models useful in pharmacological studies.
In fact, these devices are often unique and designed from time to time to meet specific study needs, making them not very versatile in terms of size and shape of the treated samples, excessively expensive to be disposable and therefore need to be cleaned and sterilized before each use. ; being usually able to house a limited number of components, they do not lend themselves to the generation of constructs in large quantities. The evolution of bioreactors in recent years has been remarkable. In the analysis conducted above, it was seen how it is possible to modify these laboratory tools, designed by researchers to carry out dynamic culture activities, adapting the components available to them to their needs in order to obtain the maximum reusable profit. Obviously, the sustainability and

accessibility to this type of tools is strictly personal and charged to the researcher but there are numerous factors that have nothing to do with bioreactors, not least the intrinsic difficulties in manufacturing the product itself and implementing the culture protocol.

The introduction of each new technical-technological tool changes the way of dealing with problems and also changes the problems that the operator considers important to solve. A versatile and multifaceted tool has a strong impact in terms of rapid prototyping and can be a strong stimulus to explore what happens in vivo, thus reducing the use of animals and increasing the effectiveness of new approach methodologies (NAM)[13].

A multidisciplinary approach, which integrates biological knowledge with engineering knowledge, those on bioprocesses with the science of biomaterials, is also essential for rapid translation and to increase the probability of success [2].

From the need to overcome some limitations derive the recent investments that biomedical research is dedicating to the development of reliable bioreactors, with sufficient characteristics for an automated, reproducible and safe production of cellular samples. These initiatives are briefly discussed in the previous sections.

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